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GIARDIA METHODS WORKSHOP

by

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16. ABSTRACT

The objectives of the workshop were to familiarize participants with the use and limitations of Giardia cyst detection methodology; to critically review the tentative Giardia method appearing in the 16th edition of Standard Methods for the Examination of Water and Wastewater; to share the experiences of the panelists and audience in working with methods for detecting Giardia cysts in water, and to make recommendations for improving the methodology.

The format for the workshop involved a brief introduction by a panel member of a specific portion of the methodology, discussion among panel members and the audience to expand various aspects of that topic and a summary and recommendations concerning that portion of the methodology. The summaries, discussions and recommendations are presented.

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INTRODUCTION

The objectives of the workshop were to familiarize participants with the use and limitations of Giardia cyst detection methodology; to critically review the tentative Giardia method appearing in the 16th edition of Standard Methods for the Examination of Water and Wastewater; to share the experiences of the panelists and audience in working with methods for detecting Giardia cysts in water, and to make recommendations for improving the methodology. Most of the panel members had participated in a workshop held in September, 1980 from which a consensus method had been developed. That consensus method formed the basis for the 16th edition tentative method.

The format for the current workshop involved a brief introduction by a panel member of a specific portion of the methodology, discussion among panel members and the audience to expand various aspects of that topic, and finally, a summary and recommendations concerning that portion of the methodology. The reader may refer to a paper by W. Jakubowski in session 2B of these proceedings for a description of the 16th edition tentative method. Additional information on Giardia immunofluorescence methods may also be found in session 2B in a paper by J. Sauch.

The summaries below were developed by the moderator from a verbatim transcript of the workshop. A serious attempt was made to make the summaries as complete and as thorough as possible, and the moderator accepts all responsibility for the accuracy of their content and editorial quality.

SUMMARIES

F. Schaefer - Sample collection, volume, transport and storage: Every water sampling situation is different, varying with whether raw or finished water is being sampled, and requiring decisions to be made about how to sample. For example, if you are sampling a raw water from a very turbid river, it is possible to collect too large a sample. Under these circumstances, the filter will contain so much silt and debris that large extract volumes will be produced (possibly in excess of 25 liters) resulting in pellet volumes exceeding 10-15 ml after concentration. Large pellet volumes create difficulties in purifying the cysts from the debris by flotation procedures. On the other hand, when sampling a filtered finished water supply, or other low turbidity water, very large volumes can sometimes be sampled with production of small pellets. As a guideline, it is suggested that the minimum sample size be 100 gallons and the maximum be 500 gallons. Another point to be stressed is the requirement for scrupulously cleaning sampling equipment in order to prevent cross-contamination between samples. Giardia cysts can be sticky and adhere to laboratory apparatus and sampling equipment. Equipment should be thoroughly scrubbed before sampling, and, if using the same filter holder to sample more than one water supply on a given trip, it should be thoroughly washed and rinsed with the next water to be sampled. The use of gloves and aseptic technique is also recommended to reduce the possibility of cross-contamination. Samples should be transported to the laboratory on ice as soon as possible. Under no circumstances should the sample be frozen. Freezing the sample can cause formation of ice crystals within the cysts, which could then result in their disruption upon thawing. Upon arrival in the laboratory, samples should be processed as soon as possible, preferably within 48 hours.

Discussion

Different laboratories are using various filter types for sample collection. Some laboratories are using yarn-wound filters of the Commercial Filter Corporation type, made from orlon or polypropylene. Other laboratories are using AMF/CUNO type filters with a cotton or polypropylene base. At least one laboratory is using the Balston epoxy fiberglass filter tube, and there has been one published report on the use of Millipore membrane filters. Most laboratories are using the depth-type filters, as opposed to membrane filters, because of the advantages afforded in filtering large volumes. No rigorous, controlled comparative evaluations of filter types have been done to determine if one filter type is superior to another. The selection seems to be based upon laboratory preference, and perception of the ease with which trapped particulates can be recovered from the filter material. At Dr. Hibler's laboratory, filters made of polypropylene, orlon or cotton are processed. They prefer the polypropylene filters because they look cleaner after washing. In addition, they can readily process 1000 gallons of "clean" western waters. Dr. Hibler also emphasized the need for cleanliness in preparation of sampling equipment, recommending the use of chlorination, soap and hot water. Participants agreed that depth-type filters should have a porosity not greater than 1 µm since some investigators have found cysts to pass through filters with nominal porosities as low as 3 µm. For processing yarn-wound filters, cutting or unwinding the yarn is a matter of personal preference.

There was some discussion on the need to regulate flow or pressure during sample collection. Some laboratories collect samples at a pressure of 15 PSI or less, and others control flow at 1 to 1-1/2 GPM. Still others use no pressure or flow regulation. With depth-type filters, high pressure can increase the nominal porosity and there is the possibility of cyst disruption through shear force. Until data become available, it would appear prudent to collect samples at the lowest feasible pressure differentials.

It is known that chlorine can distort and inactivate cysts, possibly rendering them unidentifiable. Data are now being acquired on the concentration of chlorine and contact times required to produce different degrees of distortion. This is important because it may take most of a day, if not more, to collect a single sample. Cysts collected on the filter early in the sampling could be subjected to chlorine contact for several hours, resulting in distortion that produces false negative results. There was reluctance on the part of panelists to endorse dechlorination for sampling of disinfected supplies because of the added expense, equipment and effort required to do so. However, it was agreed that chlorine can produce cyst distortion.

The question of maintaining the sampling unit filter and holder cold during sampling was raised. One panelist had occasion to sample in a warm, humid environment without access to ice or refrigeration during sampling and transport. Cysts were found, but it is not known if losses might have occurred. It would seem reasonable to keep the filter at least in the shade during sampling, and cold if possible.

Recommendations

Controlled laboratory studies are needed in order to make defensible recommendations on filter types and porosities, flow rates and pressure differentials, and transport and holding times. In the meantime, depth-type or membrane filters with a porosity of 1 µm or less, and capable of sampling the required volume, should be used. The filter composition should be of a material that will allow ready recovery of the cysts and that will not interfere with purification of cysts from the debris. Sample volumes should be a minimum of 100 gallons, and the maximum volume would depend upon the quantity of particulate matter in the sampled water. When sampling disinfected waters, dechlorination is advisable. Pressure differentials, flow rates and transport and holding times should be minimized as much as possible. Samples should be protected from sunlight and heat during collection and transport.

C. Hibler - Sample processing to concentrate state: As already mentioned we work with polypropylene, cotton and orlon filters, but we prefer the polypropylene. We split the filter to the core and hand wash it in distilled or deionized water doing as thorough a job as possible. The volume of wash water may be 0.5 to 2 gallons, depending upon how much dirt is on the filter. Formalin is added to the wash water to a concentration of 2% V/V to preserve the sample and prevent growth of other protozoa, algae, etc. The wash water is then refrigerated for 24 hours and the supernatant is siphoned down to the organic sediment. With some samples you can get down to almost nothing; with some eastern waters the organic sediment-containing portion may be greater than 200 ml. If there is a need for rapid results the wash water can be centrifuged instead of using refrigera-

tion settling. The sediment portion is centrifuged and the pellet is split if more than 1 ml of pellet is produced. The pellet is then overlayed or underlayed using zinc sulfate and centrifuged for 3 to 8 minutes. The interface material is collected and washed by centrifugation. It can then be concentrated by flotation using zinc sulfate in 15 ml tubes with a coverslip placed on the meniscus. Or it can be filtered through a 5 µm Nucleopore membrane. The material on the membrane is washed off and examined under the microscope using an iodine stain. If the debris in the sample is primarily inorganic, a zinc sulfate or potassium citrate solution with a specific gravity of 1.28 may be used to clean it up. If it is primarily organic, a specific gravity of 1.09 is used. If the debris is a mixture of inorganic and organic, a specific gravity of 1.2 is used. The important thing is to separate the cysts from the inorganic and organic debris prior to microscopic examination.

Discussion

This part of the methodology seems to be where the "art" plays a big role. There are differences of opinion, and very little data, on the medium to use for washing filters, on chemicals used in flotation procedures and on details for effecting separation of cysts from debris (e.g., underlaying vs. overlaying) and collecting the concentrate for examination (e.g., coverslip vs. recovering all of the floated phase). Some laboratories are using distilled or deionized water buffered at pH 7.0 as the washing medium and some are using potassium citrate or citrate/tween 20 combinations. The proponents of this latter method find it effective in cleaning the debris from orlon filters and say that it may help to release "sticky" cysts from filters and prevent cyst loss to glassware. When using detergent solutions or wetting agents with orlon filters, the experience has been that fewer washings and rinsings are needed in order to get the fibers clean and white to the eye. Consequently, a smaller volume of wash extract is produced and processing time is decreased if centrifugation rather than refrigeration sedimentation is used. The proponents of distilled or deionized water have not encountered sticky cysts and say that the use of detergents may interfere with recovery of cysts when centrifugation flotation is performed with a coverslip touching the meniscus and top of the tube. There did not appear to be much concern with isotonicity of the wash medium since the cyst is a resistant form and does occur in a hypotonic environment, i.e., water. However, osmotic effects do occur during flotation when cysts are exposed to the chemical solutions used in this procedure. Some prefer zinc sulfate, others prefer sucrose or potassium citrate or sucrose/percoll. All of the solutions can produce cyst distortion or changes in the specific gravity of cysts upon prolonged contact. Zinc sulfate seems to be the chemical most widely preferred by parasitologists. Percoll may exert less of an osmotic effect but it is expensive.

Recommendations

Comparative data are needed on the effectiveness of various wash media and on the suitability of different flotation chemicals. Distilled or deionized water, as recommended in the 16th edition tentative method, or phosphate buffered saline at pH 7.0 may be used as the wash medium. The addition of detergents, wetting agents or other substances should be compared and evaluated against these media. Any of the flotation chemicals listed above may be used for purifica-

tion but caution should be exercised to minimize contact time of cysts with the concentrated solutions.

E.A. Meyer - Examination of concentrate, criteria for identification, reporting results: This portion of the methdology overlaps somewhat with the previous discussion by Dr. Hibler in that examination of the concentrate involves getting the purified cysts from the centrifuge tube or membrane filter to the microscope. The tentative method calls for taking a 15 ml centrifuge tube with less than 1 ml of pellet (I would prefer less than 0.25 ml) from the sample, adding zinc sulfate at a specific gravity of 1.20 to the top of the tube and placing a clean coverslip on the meniscus. The coverslip is allowed to remain in place for several minutes to hopefully allow the cysts to stick to it. The coverslip is gently placed on a microscope slide and examined. This coverslip procedure of removing cysts from the meniscus is subject to wide variation and potential large losses of Giardia cysts. In our laboratory, we have found that the volume of meniscus material recovered on the coverslip varies from 0.25 ml to 0.75 ml. An alternative method is to recover all of the floated material with a pipet. The tentative method then suggests scanning the entire coverslip area using a 10X objective. The intent here is to recommend low power and I would suggest a total scanning magnification, including the ocular, on the order of 100X. Either bright-field or phase microscopy may be used for examination, depending upon what is available and the experience of the observer.

Concerning interfering organisms and debris, Dr. Hibler suggested some ways of dealing with the specimen when there is much inorganic or organic debris present. There are many types of organisms including yeasts, diatoms, algae and protozoa that can be confused with Giardia on the basis of size and shape. The major problem organisms are flagellated protozoa and pollens. Criteria for identification include seeing an oval organism in the size range of 8 to 19 µm and observing two or more internal morphological characteristics. I have seen Giardia cysts as small as 5 µm in length, but, generally, they are larger. With iodine stain the cyst is brownish and refractile. Lying diagonally along the length of the organism are axonemes or fibrils that are the internalized portions of the flagellae. The cysts could have up to four nuclei, usually at one end of the cyst. Median bodies are the final internal characteristic that may be observed. To identify these internal characteristics, a total magnification of 450X or greater should be used. This method cannot tell you if the cysts are viable. Finally, for reporting, it may be a good idea to photograph what you have seen and to record dimensions of organisms in the right size range with notations of internal morphological characteristics that were observed.

Discussion

One panelist suggested that it might be helpful to acclimate yourself to examination of concentrates by retuning your eye each day through observation of at least ten known cysts from a human source and recording measurements. Photography may not be too helpful in documenting the finding of a Giardia cyst because the internal morphological characteristics usually occur in several planes of focus. The quantity of concentrate pellet used in the flotation affects the recovery of cysts and recovery may drop significantly at pellet volumes of 0.5 ml and greater. The cleanliness of the coverslip is very important and even new coverslips are greasy and should be

cleaned with alcohol prior to use. For identification of Giardia, no other known organism has median bodies but it may not be possible to observe these in as much as 50% of the cysts encountered. Concerning viability, it may be possible, after gaining considerable experience, to make an educated guess that a cyst is nonviable based on its appearance, i.e., shrunken, shriveled, distorted, muddy internal characteristics, etc. However, the viability status of a cyst that appears to be morphologically excellent cannot now be determined by microscopic examination alone. There are no surrogate indicators for Giardia cysts in water, but it may be possible to determine if the type of plant debris present is of beaver or muskrat origin. Also, in reporting results, information on the effectiveness of water treatment plant filtration can be gained by examining raw and finished water. Even if Giardia cysts are not found, if there is debris in the finished water in the same size range as cysts then there is a potential problem.

Recommendations

Quantitative data are needed on the efficiency of cyst recovery when floating cysts from concentrate pellets up to 1 ml in volume. The reliability of the coverslip method versus examination of all floated material needs to be determined. Scanning of the coverslip at a total magnification of about 100X is recommended with confirmation at 450X or greater. Either bright-field or phase microscopy may be used but the investigator should become experienced by examining known Giardia cyst-containing specimens. Most cysts will be in the size range of 8 to 19 µm but it should be realized that cysts smaller the 8 µm can occur. At least two internal morphological characteristics must be observed in order to make positive identification.

G.J. Vasconcelos - Equipment requirements, analysis time, costs: Costs for sampling and processing equipment and supplies, and estimates for personnel time are shown in Tables 1-4. The sampling time will vary with the volume and type of water being sampled. Personnel time for sampling includes assembly and disassembly of the equipment. Ranges are given for the cost of sample processing equipment and examination in Table 2. These costs are estimates and were taken from recent catalogs. Personnel time listed in Table 3 is based on experience at the USEPA Region X laboratory in Manchester, WA. A range of summary costs and times is given in Table 4. Per sample costs at individual laboratories will vary with number of samples processed, quality and quantity of equipment and with labor costs.

Table 1. Sampling Costs and Time

A.	Sampling Device with Filter	$131
B.	Sampling Device with Regulator, Pressure Gauge and Backflow Preventor	$161
C.	Sampling Time (range)	
	1. Filtration time	4-24 hrs
	2. Personnel time to attach and disconnect device	1-2 hrs

Table 2. Processing and Examination Equipment and Supply Costs

A. Major Equipment	
1. High speed/capacity centrifuge	$3,200-5,000
- Swinging bucket rotor and cups	$850-3250
2. Bright-field microscope	$2,500-6,500
3. Large capacity refrigerator	$1,000-2,500
4. Hydrometers, tube mixers, stage and ocular micrometers, baths, etc.	$350-850
B. Minimal Supplies and Chemicals	
1. Expendables - plasticware, bags, slides, reagents, etc.	$500
2. Non-expendables - glassware, etc.	$570
C. Optional Equipment	
1. Microscope combinations	
- Bright-field/phase contrast	$3,750-8,500
- Bright-field/phase/FA	$6,000-12,000
- Bright-field/phase/FA/DIC	> $10,000
2. Refrigerated, high speed/capacity centrifuge w/wind-shielded swinging bucket	$5,700-10,000
3. Algal (Foerst) centrifuge	$1,500-2,000
4. Microscope camera system	$1,250-6,000

Table 3. Processing and Examination Time

A. Extraction	
1. Reference method	1-2 hrs
2. Reference method w/tween 20 and sodium citrate wash	1-3 hrs
B. Extract Concentration	
1. Refrigerator settling	24 hrs
2. Centrifuging	1-3 hrs
3. Algal (Foerst) centrifuge	(not known)
4. Processing combined sediments	
- volume = to or < 1 ml	1 hr
- volume = to or > 1 ml	2 hrs
5. Sucrose/percoll flotation w/vol >1 ml	2 hrs
C. Microscopic Examination	
1. Range per slide (of 75 examined)	10-58 min
2. Average time per slide	23 min
3. Average number of slides examined/sample	3
4. Average total time per sample	69 min

Table 4. Cost and Time Summary for Sampling and Analysis

		Low	High	With Optional Equipment
A.	Equipment and supplies	$9,100	$19,331	$28,581
B.	Personnel time/sample	5.5 hrs	11.5 hrs	
C.	Labor cost/sample at $22/hr	$121	$253	
D.	Equipment cost amortized over 5 yrs	$34	$73	$114
E.	Supplies cost over 5 yrs at 50 samples/yr	$21	$24	$33
F.	Cost per sample	$176	$350	$400

Discussion

Costs for individual orlon filters are about $4 each when purchased in case lots. Dr. Hibler's laboratory is currently charging $40 per sample for analysis if refrigeration sedimentation of the extract is used. He estimates that the total cost per sample is actually closer to about $100. A private water company representative utilizing Dr. Hibler's services estimates his costs at about $125 per sample in addition to the analysis costs.

J. Sauch - Fluorescence techniques: FA techniques offer an advantage over light microscopic examination in that, instead of looking for a bright brown object against the dull brown objects in the background, now we're looking for bright apple green fluorescing objects against a black background with perhaps, very dull yellow or red autofluorescing objects. Advantages over light microscopy are time and the ease of finding the cysts. Unfortunately, fluorescent antibody techniques require specialized equipment and reagents and an initial commitment in personnel time to develop the method and to manufacture reagents. In setting up the assay, you have to consider the antigen to make the antibody. What are you going to use for antigen? Where are you going to get cysts? The purer they are, the lower the possibility of cross-reactivity. One of the disadvantages of this method is that there is a slight possibility of cross-reactivity due to organisms that share antigens. Secondly, how are you going to prepare the antibody? What animals are you going to use? Right now, no commercial manufacturer makes Giardia antibodies. We've used rabbits to prepare antibodies because they're very easy to house in a small area, and each rabbit is capable of producing a large amount of serum with very high titer antibody. There are some manufacturers who may make the antibody for you and they may even conjugate it to do a direct type of assay. Additional considerations in using the FA/phase method described in Session 2B are the counterstain to use, the membrane filter type and how to mount the filter. The mounting media must not autofluoresce or quench the fluorescence of the conjugate. Thirdly, specialized equipment is needed. An epifluorescent microscope with vertical illumination will probably be your biggest expense. Vertical illumination is important because the illumination is only upon the section of the slide that you are looking at. This prevents bleaching of the rest of the sample.

To detect cysts in a sample, I look for fluorescing objects of the correct size and shape. I run positive controls with every assay and I've attuned my eyes to look for a certain size, shape and fluorescence with the positive controls (G. lamblia cysts). My identification procedures are based upon the size, shape and fluorescence as well as internal morphological characteristics. I use the same criteria that the reference method uses: nuclei, axonemes or median bodies upon examination by phase. It is important to use light microscopy for identification because of the potential, even though slight, for cross-reactivity. Negative controls should also be run to guard against sample cross-contamination or laboratory contamination of glassware or equipment. The time required to prepare the concentrate for examination by FA is about 1.5 hours. Depending upon the quantity of particulate matter in the sample, it could take anywhere from 20 minutes to examine a very clean sample to a couple of days for a very dirty water sample.

Discussion

In the FA/phase technique, FA is used to detect cyst-like objects and confirmation of identity is made by switching to phase microscopy and observing the internal morphologic characteristics. The condition of the cyst cannot be determined by FA alone and identification is not based solely on the FA reaction. Organisms which fluoresce under FA but in which internal characteristics cannot be discerned are reported as "Giardia cyst-like objects". Cysts exposed to chlorine at high dosage levels or for long contact times will react with FA reagents and glow under UV, but if the internal morphology cannot be seen under light microscopy, the sample will not be reported as Giardia-positive. When using FA techniques, additional time is required to prepare the specimen for examination under the microscope. However, the level of expertise required to detect cysts, as well as the amount of time required to scan the specimen, may be reduced. The actual amount of time involved in using either the tentative method or FA techniques will vary from laboratory to laboratory with the experience and training of the personnel. Available microscopic detection and identification techniques, including FA, do not distinguish living from dead cysts. The USEPA is currently funding work to develop metabolic stains for viability that might be used in conjunction with FA techniques.

Recommendations

FA techniques for Giardia are in an early stage of development. Additional work is needed to characterize Giardia antigens; to develop guidelines for antibody production, purity and use, and to develop criteria for cyst identification.

J. Riggs - Antibody identification, species differentiation: We first worked with polyclonal antibody which is produced when you inoculate an animal with an antigen, in this case, whole cysts from a human source. The antibody is recovered from the serum of the inoculated animal, labelled or conjugated with a fluorescent dye and used in the fluorescence technique. Polyclonal antibodies leave a lot to be desired because, if the inoculated animal has an antibody against an organism that is in, for example, a filter from a water system, the antibody will react to that and produce fluorescence. The purity of the antigen inoculated into the animal is another source of background fluorescence. It is actually a specific fluorescence

because the animal will make antibodies against bacteria or other organisms or proteins that are in the "purified antigen". What we have done is to produce monoclonal antibodies against an antigen or antigens in the Giardia cyst. Monoclonal antibodies are produced through a tissue culture procedure using mouse hybridoma cells. In this procedure, single cells that produce antibodies to specific antigens in the Giardia cyst can be isolated and cloned. These cells can be grown in culture and they will continue to produce the antibody that the cell was first primed against. We have used monoclonal and polyclonal antibodies to try to separate human Giardia from non-human Giardia. We prepared monoclonal antibody against human-source cysts and conjugated the antibody with rhodamine, a red fluorescing dye. We reacted the monoclonal antibody with a mixture of human and mouse-source cysts. Only the human-source cysts reacted with the monoclonal antibody and they stained red. After the reaction with monoclonal antibody had taken place, we washed the excess antibody away and then reacted the mixture of cysts with a polyclonal antibody conjugated with fluorescein, a green fluorescing dye. The polyclonal antibody couldn't react with the human-source cysts because they were already covered with monoclonal antibody. But the polyclonal antibody could react with the murine or non-human type cysts and they stained green. This allows us to differentiate and say that in this specimen both human and non-human Giardia cysts were present.

Discussion

The monoclonal antibodies produced by Dr. Riggs' laboratory were developed from cysts obtained from a single human stool specimen. His laboratory has looked at over 1,000 human stool specimens and the monoclonal antibody reacted with all of them. Specimens from two beavers have been examined and one was positive with both the polyclonal and monoclonal antibodies indicating that the beaver was infected with human and non-human type Giardia. It is not known if this monoclonal antibody would react with all human-source Giardia or if it would react with all cysts in a given specimen. There appears to be a biological difference in cysts from different human sources in that some will produce infection in Mongolian gerbils with resultant cyst excretion while others will cause infection in gerbils but without cyst production. Even with cysts from the same donor, there is variability in the percent of cysts that will excyst. Panelists cautioned that parasites may be subject to antigenic modification dependent upon the host. Panelists also suggested that monoclonal antibodies be developed using cysts from different human and animal sources and that they be cross-checked against each other. Dr. Riggs indicated that his laboratory is in the process now of trying to produce monoclonal antibodies against cysts from other sources.

Recommendations

Additional groundwork is needed with respect to both polyclonal and monoclonal antibodies including characterization of cyst antigens, production of cysts in culture and standardization of cyst preparations and reagents. Antibodies should be produced to cysts from a variety of human and animal sources and thoroughly evaluated before their use in identifying species of origin can be validated.

G. Healy - Quality control, performance evaluation, laboratory certification: Quality controls in parasitology are less defined and less rigid than they are in other areas of microbiology. Reliable diagnosis

is based on accurate identification of organisms and competent performance of techniques for recovering and demonstrating the organisms. Proper quality control involves:

1. Trained Personnel - minimum of 150 hours of lectures and laboratory, periodic refresher courses, participation in proficiency testing program(s).

2. Equipment - microscopes, centrifuges, refrigerators, fume hood, slides, coverslips, stains, solutions.

3. Use of Appropriate Techniques - wet mounts, concentrates, stained slides.

4. Reference Materials - manuals, books, library of well-defined specimens from commercial or professional courtesy origin.

5. Reference Collection - maintaining a number of organisms in formalin and/or polyvinyl alcohol fixative.

6. Periodic Use of Reference Material for Accuracy of Routine Performance.

In 1975, because of some severe problems in diagnosis in New York City, the Congress passed the Clinical Laboratory Improvement Act, which mandated that laboratories had to have a certain level of proficiency. The College of American Pathologists, the American Association of Bioanalysts and the Centers for Disease Control are the three principle proficiency testing groups. In clinical parasitology, the situation is different than it is for water bacteriology or water microbiology in that everything is done by consensus. Specimens are sent out to a number of laboratories that are identified as reference or referee laboratories. The performance of other laboratories is then compared against what the reference laboratories have reported to be present in the specimens. Proficiency testing and performance evaluation are going to be very important in the area of water parasitology not only because of Giardia. Cryptosporidium is another protozoan intestinal parasite that may be transmitted through drinking water. The question of laboratory certification is another complex problem even in clinical parasitology, but it is something that also needs to be addressed in environmental parasitology.

Discussion

QA/QC, performance evaluation and laboratory certification are important topics in water parasitology and the need for them will probably increase. The initial focus should be on assessing qualitative performance since quantitation of cysts with available methods is very difficult and open to question. Detecting and identifying cysts is one problem and determining the viability of detected cysts is yet another problem. QA/QC will be needed as well for viability determinations. Recently developed animal models include the Mongolian gerbil and suckling mice but much additional work needs to be done to determine infective dose, conditions for establishing infection and the ability of cysts from different sources to routinely produce infection in animal models. Training is another area that needs to be addressed. The USEPA does not conduct formal training programs in Giardia methods. Since so many of the procedures are more art than science, hands-on workshops might be advisable. These

may be conducted under the auspices of the American Society for Microbiology or through some other suitable mechanism. Alternatively, videotape or slide presentations may be of value.

Recommendations

Steps should be taken to form work groups or committees to develop QA/QC, performance evaluation and laboratory certification guidelines in the area of water parasitology, and to develop a training program in Giardia methods.

Concluding Discussion

At the conclusion of the workshop, it was re-emphasized by a participant from Colorado that a negative finding of Giardia using currently available methods does not mean that a given water supply is safe or not at risk. This is true because of variability in method efficiency and because a stream or water supply might be negative on the day of sampling and positive on a day when a sample was not collected. More useful information might be provided by looking at the differential in particle numbers and sizes between raw and filtered waters to determine the efficiency of treatment. It was agreed that there are better ways to monitor the efficiency of filtration than by performing a Giardia analysis. However, there are many unfiltered water supplies in the United States and until and unless a regulation is promulgated requiring the filtration of all surface water supplies, there apparently is a need for a method to specifically detect Giardia cysts and not just particulates in the same size range. The USEPA does not require monitoring for Giardia and the methods work that has been done is related to assisting in outbreak investigations and gathering data on occurrence and health effects of the organism.

ACKNOWLEDGEMENTS

This was a long workshop but the lively interest and active participation of the audience made the time go all too fast. To our audience, we extend our sincere appreciation for making this a successful workshop.

I would like to acknowledge AWWA for allowing us the opportunity to conduct this workshop as part of the Water Quality Technology Conference and especially Jack Cleasby, who was instrumental in getting it on the program, and our thanks to Rick Harmon and Dave Hubley for helping out in the taping and monitoring of the session.

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